II. Rejection under 35 U.S.C. 112, first paragraph

Claims 43-47 are rejected under 35 U.S.C. 112, first paragraph as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Applicants traverse the rejection.

A. Summary of the Presently Invention

Alzheimer's disease is a progressive disease resulting in senile dementia. The human disease is characterized by the presence of insoluble protein aggregates (plaques) visible by microscopic analysis of sections of brain tissue. The principal constituent of the plaques is a peptide termed β-amyloid peptide. β-amyloid peptide is an internal fragment of a precursor protein termed β-amyloid precursor protein (APP). There is general agreement that Alzheimer's disease results, at least in part, from processing of APP to generate β-amyloid peptide. Several mutations within the APP protein, including the Swedish mutation, have been correlated with the presence of Alzheimer's disease. One of the enzymatic activities that processes APP to β-amyloid peptide is referred to as β-secretase activity. This activity generates the free N-terminus of β-amyloid peptide.

The presently claimed invention, as defined by claim 42, is directed to a method for screening a compound to determine its ability to alter the amount of an Aβ (x-≥ 41) peptide in a cerebral spinal fluid ("CSF") sample from a non-human animal models that expresses amyloid-β precursor protein (APP) in the brain and processes it to one or more soluble Aβ peptides. Dependent claim 43 specifies that the model is a rodent model, and claim 44 specifies that the model is a mouse model. Dependent claim 45 specifies the non-human animal model is a transgenic animal model having an expression cassette that drives the expression of a sequence which encodes the Swedish mutation of an APP gene. The Swedish mutation results in substitution of wildtype residues at positions 595 and 596 with asparagine and leucine respectively. The transgenic animals express the transgene to produce Swedish APP. Claims 46 and 47 depend from claim 45.

Claim 46 specifies that the model is a rodent model, and claim 47 specifies that the model is a mouse model.

The presently claimed methods of screening do NOT require that the animals exhibit cerebral deposition of Aβ. The presently claimed screening methods can be performed whether or not cerebral deposition of Aβ occurs in an animal model. The presently claimed screening methods result at least in part from the discovery that the cerebral spinal fluid ("CSF") of individuals suffering from Alzheimer's disease generally contains Aβ (x-≥ 41) in amounts which are below the average range present in the CSF on the non-Alzheimer's individuals. (*See* page 11, lines 30-35 of the specification.) The application discloses a screening assay in which compounds are administered to non-human animal models, and the compound's capacity to increase, decrease, or leave unchanged the amount of soluble Aβ (x-≥ 41) in a CSF fluid sample is measured (*see* page 24, lines 5-20). Compounds which increase the amount of soluble Aβ (x-≥ 41) in the CSF are candidates for treating Alzheimer's disease; and, compounds which decrease the amount of soluble Aβ (x-≥ 41) in the CSF are compounds likely to hasten Alzheimer's disease. (*See* page 24, lines 5-17 of the specification.) Humans, guinea pigs, and dogs have been shown to have Aβ (x-≥ 41) in the CSF. (*See* Parts 3 and 4 of the Experimental Section of the specification.)

The provision of screening methods that do not rely on cerebral deposition of Aβ can be advantageous. Compounds can be screened by following the β-amyloid precursor protein (APP) processing reaction in CSF samples from animal models. Thus, the presently claimed screening method is in fact simpler and quicker than attempting to detect plaques in tissue sections by microscopy as is required to perform certain other screening methods.

B. Claims 43-44

It is the position of the Office Action that the claims 43 and 44 are not enabled because (1) the specification fails to teach rodent or mouse models that exhibit cerebral deposition of Aβ for use in the presently claimed screening assay, and, (2) it was

well established in the art at the time of filing that rodents did not deposit Aβ in the brain. Claims 43 and 44 depend from claim 42. Claim 42 has been amended so that it no longer refers to cerebral depositions of Aβ. Thus, the Office Actions specific concerns with an animal model having a phenotype which includes cerebral depositions of Aβ are moot.

The non-human transgenic animal model as presently claimed in claims 43 and 44 displays the phenotype of an APP gene, but does not require the phenotype of cerebral deposition of Aβ. It is noted that the Office Action has not alleged that making a transgenic animal that expresses APP would require undue experimentation. As discussed below, U.S. Patent 5,604,102 (which the instant specification incorporates by reference) provides a viable animal model for elucidating the processing of βAPP into βAP and related fragments and further provides a convenient system for screening for inhibitors of β-secretase activity and/or for drugs that modulate β-secretase activity.

Based on the foregoing, Applicants request the rejection of claims 43 and 44 under 35 U.S.C. § 112, first paragraph be withdrawn.

C. Claims 45-47

It is the position of the Office Action that the claims 45-47 are not enabled because (1) the specification fails to provide an enabling disclosure; and, (2) lacks an adequate written description on how to make non-human transgenic animals (including rodents or mouse) that specifically exhibit cerebral deposition of Aβ by means of an expression cassette that drives expression of a sequence which encodes the Swedish mutation of a APP gene for use in the presently claimed screening assay.

Claims 45-47 depend from claim 42. As discussed above, claim 42 has been amended so that it no longer refers to cerebral depositions of Aβ. Thus, the Office Actions specific concerns with an animal model having a phenotype which includes cerebral depositions of Aβ are moot.

Claim 45 is directed to the use of a transgenic animal model having an expression cassette that drives expression of a sequence that encodes the Swedish mutation of an APP gene. Claims 46 and 47 depend from claim 45, and are directed to

the use of a rodent model or a mouse model, respectively. The Office Action states that the phenotype of the non-human animal is a critical claimed feature of the animal model as claimed. The non-human transgenic animal model as presently claimed in claims 45-47 displays the phenotype of the Swedish mutation of an APP gene, but does not require the phenotype of cerebral deposition of Aβ.

U.S. Application No. 08/143,697 Issued as U.S. Patent 5,604,102 Teaches the Generation of Transgenic Animal Models Expressing the Swedish Mutation of an APP Gene

U.S. Patent 5,604,102 provides evidence that as of the time of filing the instant application transgenic animal models expressing the Swedish mutation of an APP gene could be reliably produced. The specification as filed incorporates U.S. Application No. 08/143,697 filed October 27, 1993 by reference. U.S. Application No. 08/143,697 issued as U.S. Patent 5,604,102. Applicants note the Amendment filed August 7, 1997 amended the specification to replace "U.S. patent application 08/143,697" with "U.S. Patent 5,604,102." Therefore, incorporation by reference is proper.

Part 6 of the Experimental section of U.S. Patent 5,604,102, attached hereto, teaches generating transgenic mice using plasmids containing the 751 form the βAPP. The founder mice thus generated were screened for expression of human βAPP by analysis of their F1 offspring, which demonstrated expression of βAPP. (*See* col. 15, line 26 to col. 16, line 13.

U.S. Patent 5,604,102 provides a viable animal model for elucidating the processing of βAPP into βAP and related fragments. The Patentee verified the utility of the approach as an animal model as follows:

> In order to verify the utility this approach as an animal model, soluble fractions of transgenic animal brains were probed for the presence of the "92" form of the secreted βAPP (FIG. 8). This form is produced as a byproduct of the production of βAP and inhibition of the production of this form in cultured cells accompanies inhibition of the cleavage of the N-terminal end of βAP, the site cleaved by β-secretase.

> Brains from transgenic (Swedish Hillary 14) or non-transgenic mice were homogenized in 50 mM Tris 10 mM EDTA together with the above described protease inhibitor cocktail and centrifuged at 55K rpm for 10 min as described above. The supernatant was analyzed by Western blot utilizing the Swedish "192" antibody that reacts only with the secreted form of βAPP produced by β-secretase. For Western analysis proteins were separated on a 6% SDS PAGE gel (from Novex) and then transferred to immobilonP by standard techniques. The filter was incubated with 2 μg/ml of the Swedish "192" antibody using standard techniques, and the bound antibody visualized using the AmershamECL kit. As shown in FIG. 8, lane 3, there was robustly detectable "192" reactive material in the supernatant from the transgenic animal. The non-transgenic animal brain homogenate contained a low amount immunoreactive material that is slightly faster in mobility on the gel than the material specific to the transgenic animal (lane 2). This material is probably not related to βAPP since it does not hybridize with other βAPP antibodies (e.g. anti-5).

(*See* col. 18, line 44 to col. 18, line 4).

U.S. Patent 5,604,102 further provides a system which use the animal model to screening for inhibitors of β-secretase activity and/or for drugs that modulate β-secretase activity. Claim 1 recites:

> A method for monitoring β-amyloid precursor protein (βAPP) processing in vivo, said method comprising specifically detecting the presence of Swedish variant amino terminal fragment of βAPP (ATF-βAPP) in a specimen from rodent transformed to express the Swedish mutation of human βAPP, wherein the amino terminal fragment has been cleaved between Leu^{596} and Asp^{597}.

(*See* claim 1 of U.S. Patent 5,604,102)

U.S. Patent 5,604,102 further states, "[i]t will be appreciated that the preparation of other transgenic animals expressing the Swedish human βAPP may easily

be accomplished, including rats, hamsters, guinea pigs, rabbits, and the like." (*See* col. 10, lines 7-10.)

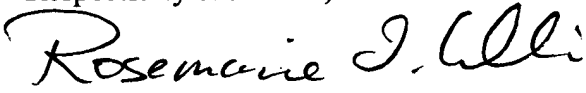
Based on the foregoing evidence, Applicants request the rejection of claims 45-47 under 35 U.S.C. § 112, first paragraph be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,



Rosemarie L. Celli
Reg. No. 42,397

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (650) 326-2400
Fax: (650) 326-2422
RLC
PA 3171596 v1

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Please amend claim 42.

42. (Thrice amended) A method for screening a compound to determine its ability to alter the amount of an Aβ (x-≥ 41) peptide in a cerebral spinal fluid sample comprising:

measuring a first amount of one or more soluble Aβ (x-≥ 41) peptides in the cerebral spinal fluid sample of a non-human animal model that [exhibits cerebral deposition of Aβ]expresses amyloid-β precursor protein (APP) in the brain and processes it to the one or more soluble Aβ peptides;

administering the compound to the non-human animal model;

measuring a second amount of [said]the one or more soluble Aβ peptides in the cerebral spinal fluid sample of the non-human animal model; and

comparing the first amount with the second amount,

the difference indicating whether the compound increases, decreases, or leaves unchanged the amount of soluble Aβ (x-≥ 41) in the cerebral spinal fluid sample.